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Rapid Synthesis and Characterization of Silver Nano Particles by Novel *Pseudomonas* sp."ram bt - 1"

Muthukannan Rammohan[1], Karuppiah Balakrishnan[2*]

[1]Department of Biotechnology, K.S.R. College of arts and Science,Tiruchengode -637215.Tamilnadu,India.
[2]Department of Immunology, School of Biological Science, Madurai Kamaraj University,TamilNadu,India.

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*Corresponding Author

Tel : +91452 2458269
Fax : +91452 2458449

Email:
immunobala@rediffmail.com

Abstract

The usage of micro organism for the production of Bio – metallic Nano particles is the promising field of biotechnology and their potential .applications from agriculture to medicine are Inevitable. In the present study, we reported that the Screening and molecular identification of a novel strain psuedomoans sp. ram bt-1 from marine sample (Coast of Bay of Bengal) through 16s rRNA ribotyping (Gen bank accession number:GU 591788) .
Synthesis of silver nanoparticles by this new strain at the intracellular level has been charecterized by UV-Spectrophotometer,X-Ray diffraction, and Scaniing electron microscope analysis.SEM observations are revealed that silver nano particles are having poly dispersed and different sizes are ranging from 20 to 100 nm in size.
(XRD) results have shown that these nanoparticles exhibit a face-centered cubic crystal structure. To the best of our knowledge, this is the first report of intracellular rapid production of silver nanoparticles are synthesized and characterized by marine strain Pseudomonas sp.

Key Words: Pseudomonas sp ram bt-1, Silver nanoparticles, Uv-Spec,XRD and SEM

Introduction

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape and controlled disparity. Many biological organisms, both unicellular and multicellular, are known to produce inorganic materials either intra or extra-cellularly (1) often of nanoscale dimensions and of exquisite morphology and hierarchical assembly.

Thus, there is a need for green chemistry that includes a clean, non toxic and environment friendly method of nanoparticles synthesis [2]. Some well-known examples of bio-organisms synthesizing inorganic materials include magnetotactic bacteria (which synthesize magnetite nanoparticles) [15] and actinomycetes like extremophilic actinomycete, Thermomonospora sp. [16] and alkalotolerant actinomycete Rhodococcus sp. [17]. Both live microorganisms and dead microorganisms are gaining importance by virtue of their facile assembly of nanoparticles. Gold particles of nanoscale dimensions may be readily precipitated within bacterial cells by incubation of the cells with Au3+ ions [18]. Bacterium Pseudomonas stutzeri AG259 isolated from a silver mine, when placed in a concentrated aqueous solution of AgNO3, resulted in the reduction of the Ag+ ions and formation of silver nanoparticles of well-defined size and distinct morphology within the periplasmic space of the bacteria [19]. silver [22-24]. Several attempts of synthesis of metal nanoparticles have been made by researchers. Synthesis of gold nanoparticles within live alfalfa plants by gold uptake from solid media has been reported [25]. They also showed the biosynthetic procedure of silver nanoparticles by alfalfa sprouts [26]. It has been reported that the Au (III) ions are reduced in the solid media to Au (0) by the plant and then the atoms are absorbed into the plant where the nucleation and growth of gold nanoparticles takes place. This method can be very efficient in decontaminating soil polluted with heavy metal ions.

Here we reported a Rapid synthesis and characterization of silver nanoparticles are occurring at intracellular of marine strain pseudomonas sp. ram bt-1. On treatment of aqueous solution of 1mM silver Nitrate with bacterial strain, silver nanoparticles could be rapidly fabricated at intracellular level and was characterized by UV-Vis spectroscopy, XRD, SEM analysis. It is revealed that the silver nanoparticles are monodisperse and with different morphologies as spherical to triangles ranging from 20 to 100 nm in size.

Experimental

Identification and screening of Bacteria from marine Coast.

Bacterial strain was isolated from marine coast. Bacterial strain was isolated by serial Dilution up to 10^9. Gram staining Proves it as a Gram negative .

PCR and Sequencing.

From the bacterial culture genomic DNA extraction have done follwed by PCR ,cloning and Sequencing of 16s rRNA amplified product with 16s rRNA primer. The DNA pellet was resuspended in sterile distilled water. Eubacterial specific primers (forward Primer 24f-5 'AGAGTTTGATCCTGGCTCAG 3 ') and (reverse primer 1492-5' ACGGCTACCTTGTTACG ACTT 3') were used to amplify 16S rRNA genes.

PCR fragments were purified using GFX TM PCR DNA and Gel Band Purification Kit (Amersham Biosciences, New

Jersey, USA). The 16S rDNA amplicon was cloned in pTZ57R/T Vector according to the manufacturer's instruction (InsT/AcloneTM PCR Product Cloning Kit #K1214, MBI Fermentas). Full length sequencing of the rRNA gene (about1,500bp) from the bacterial single colony was carried out in Macrogen (Seoul, Korea). The full-length sequence was obtained and matched with the nearest relatives of each organism by BLAST searches (Altschul et al. 1997).

Preparation of silver nanoparticles.

500ml culture of bacterial biomass was spun down; carefully weighted 0.5 gm biomass was added to 100 ml of 1 mM aqueous AgNO3 solution, in 250 ml conical flasks and kept at 30^0C in a shaker.

UV-Vis spectroscopic studies

The silver Nanoparticles start synthesis and Uv-Vis spec reading was taken at the range of 280 – 520 nm.The bioreduction of Ag+ in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the samples with 2 ml of

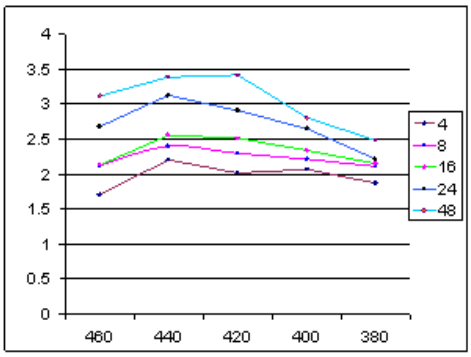
De ionized water and subsequently measuring UV–vis spectra of the resulting diluents. UV–vis spectroscopy analyses of nanoparticles produced were carried out as a function of bio reduction time at room temperature on ELICO UV spectrophotometers at a resolution of 1 nm.

X-ray diffraction measurements

X-Ray diffraction (XRD) measurements of the bio reduced silver nitrate solution drop-coated onto glass substrates were done for the determination of the formation of Ag nanoparticles.

SEM observations

Samples of the aqueous suspension of silver nanoparticles were prepared by placing a drop of the centrifuged suspension on carbon-coated copper grids and allowing water to evaporate. SEM observations were performed on an H-600 electron microscope (Hitachi, Japan) operated at an accelerating voltage of 120 kV.



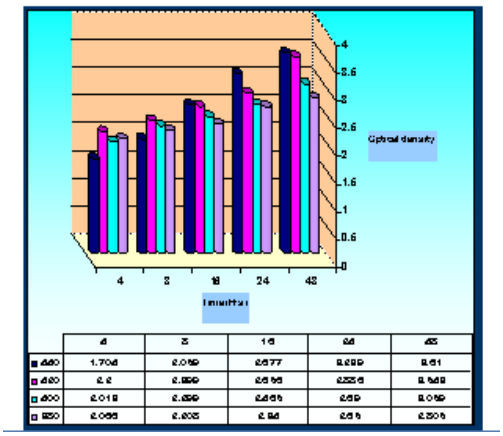
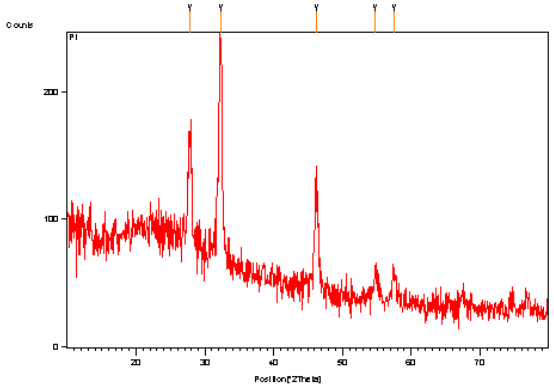


Fig .2 & 3 Graph and bar diagram explaining the silver nitrate synthesis is related to maximum production occur at 48 Hours and UV/VIS Spec reading.

Results

Fig .1.Image shows, 1 mM Ag no3 aqeuous soln is treated with Cell biomass and D.D H20 used as a Control .Observation was taken at 48 hrs.



Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
27.7773	89.94	0.7073	3.20910	49.46
32.2054	181.85	0.5788	2.77725	100.00
46.2144	85.01	0.8293	1.96278	46.75
54.7724	23.39	1.2320	1.67461	12.86
57.4954	19.78	1.3897	1.60161	10.88

Fig .4 The sample was drop-coated onto aluminum plate by just dropping a small amount of sample on the plate frequently, allowed to dry and finally thick coat of sample was prepared. The XRD measurement was performed on a Shimazdu, model LabX-XRD-6000 instrument operated at a voltage of 20 to 30 keV and a current of 30 mA with Cu K α radiation with a wavelength of 1.5418 Å.

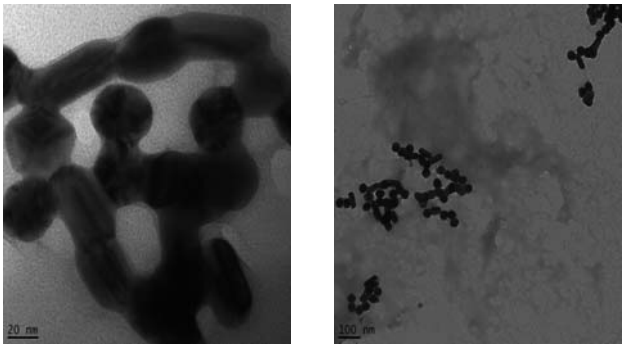


Fig - 5 SEM image has shown individual silver particles as well as a number of aggregates. The morphology of the silver nanoparticles was predominately spherical and aggregated into larger irregular structure with no well-defined morphology observed in the micrograph.

Discussion

In this work, a marine bacteria was used for synthesis of silver nanoparticles.It is well known that silver nanoparticles exhibit brown color in water; this color arises due to excitation of surface plasmon vibrations in the metal nanoparticles. The biomass incubated with deionized water (positive control) retained its original color i.e. yellow-green, while the silver nitrate treated biomass turned dark red (as shown in Fig.1a) after 6 hours due to the formation of silver nanoparticles intracellular. This shows that it was a fast process. This color is primarily due to the surface plasmon resonance of silver nanoparticles. In case of negative control (silver nitrate solution alone), no change in color was observed and the silver nanoparticles analyzed by UV-Vis spectra and SEM were stable after 4 month. Biological systems, masters of ambient condition chemistry, synthesized nanomaterials that are hierarchically organized from the nano- to the macroscale.The surface Plasmon resonance (SPR) band for spherical silver nanoparticles occurs in the range 380-440 nm. Graph shows the evolution of the absorbance spectra emanating from silver nanoparticles over time manifests increasingly sharp absorbance with increasing time of reaction at around 430 nm attributed to the surface plasmon resonance band (SPR) of the silver nanoparticles. While no absorption band was observed in both controls (Positive and Negative). After 96 h of incubation, no change in intensity at 430 nm was observed indicating the complete reduction of silver ions .

The sharpening of the peaks clearly indicates that the particles are in the nanoregime. The average size of the silver nanocrystallites as estimated from 20nm -100nm as per the report was observed in SEM analysis. Eukaryotic organisms such as fungi may be used to grow nanoparticles of different chemical compositions and sizes. A number of different genera of fungi have been investigated in this effort and it has been shown that fungi are extremely good candidates in the synthesis of gold nanoparticles[20, 21].

The silver nanoparticles formed were polydisperse, predominantly spherical with some nanotriangles in the range of 20-100 nm, some small particles in the regime of 10-20 nm are also present. It may be noted that the value obtained from SEM is in very good agreement with that obtained from XRD measurements. Under careful observation, it is noted that the some silver nanoparticles are present as clusters which explains the appearance of a longitudinal component in the UV-Vis spectra recorded from the silver nanoparticles solutions as discussed earlier (Fig.1& 2). The assembly of the some silver nanoparticles.The biosynthetic method employing plant extracts has received some attention as a simple and viable alternative to chemical procedures and physical methods synthesizing metal nanoparticles only in recent years. Sastry et al aforementioned attained the biosynthesis of metal nanoparticles by plant leaf extracts and their potential applications [27-29].

Conclusion

In conclusion, it has been demonstrated that the Psudomonas sp. ram bt-1 is capable of producing silver nanoparticles by intracellular and silver nanoparticles are quite stable in solution for months. This is one of the novel and fast approach for the production silver nanoparticles. from marine bacterium Psuedomonas sp.ram bt-1 is an efficient, eco-friendly and simple process. We are focusing to prepare mono dispersed nanoparticles by controlling several parameters as concentration of $AgNo_3$ and/or the content of biomass can be optimized for the production of Silver nanoparticles of required shapes and sizes. This study highlights the use of marine Bacterium for the production of nano particles by a rational biosynthetic Procedure.

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